Occurrence of the Parasite *Ergasilus intermedius* (Copepoda: Ergasilidae) on the Gills of Macquarie Perch, *Macquaria australasica* (Percichthyidae)

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The parasitic cyclopoid copepod *Ergasilus intermedius* is recorded for the first time on the threatened Macquarie perch, *Macquaria australasica*, which represents the most southerly record of this parasite. Infestations (up to 50 parasites per gill arch) have been observed on broodfish (310–360 mm total length, 270–750 g weight) held in earthen ponds; 47% of fish examined in 1987 and 24% of fish examined in 1988 were infested. Attachment of *E.intermedius* to the gills induced chronic, segmental branchitis, characterised by localised epithelial hyperplasia, lamellar fusion and mucous metaplasia adjacent to the constriction caused by the clasping antennae. Infested fish held in 2000 L tanks were successfully treated with a single application of 2.0 mg/L Dipterex® for 24 hours. Lower dosages of Dipterex (0.15 and 1.5 mg/L) and other chemicals, such as NaCl and NaCl plus methylene blue in tanks, and malachite green in ponds, failed to eradicate the parasite. Discrepancies in the identity and source of the type host of *E. intermedius* are discussed. Potential implications for *M. australasica* conservation programs are briefly outlined.

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KEYWORDS: Australian freshwaters, conservation, Murray-Darling river system, parasitic cyclopoid copepod, pathology, treatment.

INTRODUCTION

The cyclopoid copepod *Ergasilus* is an important ectoparasite of wild and farmed fish around the world (Amlacher 1970; Kabata 1970; Sarig 1971). In Israel, for example, over 150 ergasilid parasites per gill filament have been recorded on farmed mullet, *Mugil* spp. which have caused massive mortalities (Sarig 1971). In Australia, ergasilids have been reported from marine, estuarine and freshwater fishes (Cressey and Collette 1970; Roubal 1981; Byrnes 1986; Kabata 1992). Much of the lifecycle of ergasilids is spent in a non-parasitic planktonic phase. Only mature females are parasitic, attaching to the host following copulation, whereas males are non-parasitic (Kabata 1970).

The Macquarie perch (*Macquaria australasica* Cuvier) is a freshwater fish native to south-eastern Australia. The present distribution of Macquarie perch is restricted to small isolated populations in some tributaries of the Murray River system and some river systems of the South-East Drainage Division (Cadwallader 1981). Consequently, the species is classified nationally as vulnerable (Jackson 1998) and is the subject of several conservation management programs (Ingram et al. 1990).

During captive breeding trials at NSW Fisheries' Inland Fisheries Research Station (IFRS), Narrandera, *Ergasilus intermedius* Kabata was identified on Macquarie perch

broodfish. This paper describes the occurrence of *E. intermedius* on Macquarie perch, including observations on the pathology associated with attachment and treatment of infestations. In addition, discrepancies in the identity and source of the type host for *E. intermedius* are identified and discussed.

MATERIALS AND METHODS

Occurrence and identification

Macquarie perch broodfish, which were collected from the wild, were maintained in earthen ponds at the IFRS. The source of these fish and conditions under which they were maintained were outlined in Ingram et al. (1994).

Between September to November each year, when the maximum water temperature measured near the bottom of the ponds was between 15°C and 21°C, broodfish were transferred to 2000 L tanks in the hatchery for breeding trials (described in Ingram at al. 1994). Each fish was anaesthetised with 0.1 g/L benzocaine (Sigma), and their total lengths, weights and general condition were recorded. After breeding trials, fish were returned to the ponds.

The gills of fish were examined macroscopically and individual gill filaments with attached parasites were removed and fixed in 5% phosphate-buffered formol-saline, dehydrated in alcohol and embedded in paraffin wax. Longitudinal and transverse sections, 5µ thick, were stained with haematoxylin and eosin and examined histologically. Whole preserved parasites were mounted on microscope slides in polyvinyl lactophenol (containing acid fuchsin) and identified using descriptions provided by Kabata (1992).

Treatment of infestations

In order to eradicate the ergasilids, infested fish were treated with a range of chemicals commonly used to control external parasites (Rowland and Ingram 1991). Chemicals and dosages applied to 2000 L tanks containing infested fish were 10.0 g/L NaCl (60 min.), 5.0 g/L NaCl plus 1.0 mg/L methylene blue (24 hour), 0.15 mg/L Dipterex® (0,0–Dimethyl 2,2,2–trichloro-1–hydroxyethyl phosphonate) (Bayer) (24 hours) and 1.5 mg/L Dipterex® (24hours). In addition, earthen ponds containing Macquarie perch broodfish were treated with 0.08 mg/L malachite green (Bayer).

RESULTS AND DISCUSSION

Parasite occurrence and pathology

E. intermedius was found on the gills of Macquarie perch broodfish during spring in two consecutive years, 1987 and 1988. *E. intermedius* was not recorded from other species of fish held at the IFRS.

In 1987, 14 of the 30 fish (47%) examined were infested. Infestations ranged from less than one to 50 parasites per gill arch. In 1988, fewer fish were infested and the intensity of infestation was lower; 7 of 29 fish (29%) were parasitised, with 0–10 (mean 2.25) parasites per gill arch. Infested fish were 310–360 mm (mean 314 mm) in total length and 270–750 g (mean 492 g) in weight. *E. intermedius* were attached 0.63–0.94mm (mean 0.77mm) from the tip of the gill filaments. The second antennae of the ergasilid encircled the gill filament causing constriction of tissue at the site of attachment (Fig. 1).

The branchial epithelium at the site of attachment was hyperplastic and had formed a cushion of proliferated epithelial cells adjacent to the site of constriction (Fig. 2). Adjacent

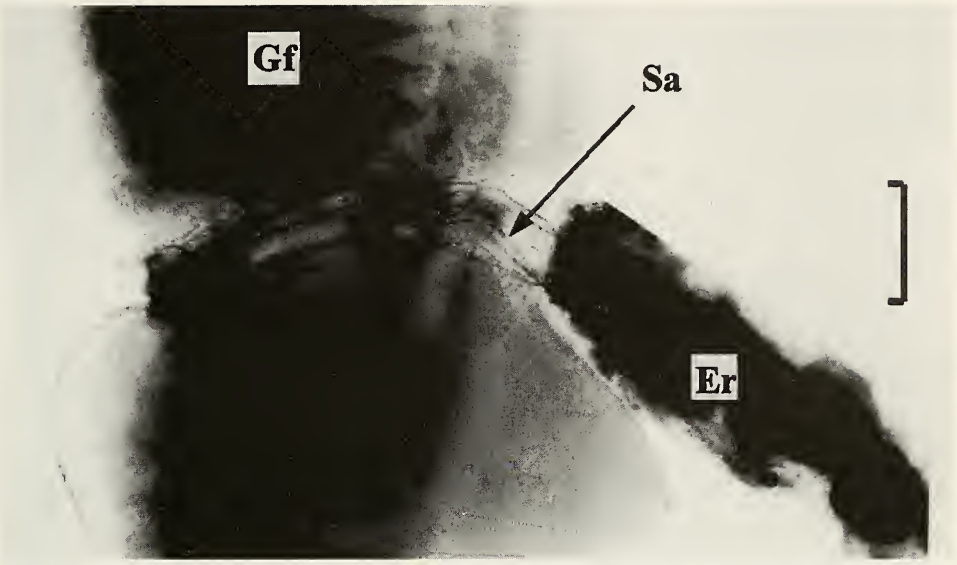

Figure 1. *E. intermedius* (Er) attached to the gill filament (Gf) of Macquarie perch. Arrow indicates second antennae (Sa) encircling the gill filament. (Scale bar length = 0.20 mm).

gill lamellae were fused. Epithelial cells in the cushion were polyhedral in deeper layers, but became more flattened superficially. The epithelium was vacuolated, oedematous and exhibited mucous metaplasia. Several cystic spaces had formed. Small numbers of lymphocytes and eosinophilic granular cells infiltrated the deeper layers of the epithelium (Fig.3).

The histological changes in the gills at the site of attachment of the ergasilid probably represented a localised reaction to irritation from the feeding of the parasite (Oldewage and van As 1987), as well as constriction of the epithelium by the modified antennae. Ergasilids secrete enzymes which digest tissues of the host externally, facilitating the ingestion of particulate material, and which may also contribute to the pathological effects of the parasite (Smith 1975; Oldewage and van As 1987). However, Roubal (1986) suggested that the effects of attachment were more damaging than those of feeding. Pathiratne (1992) attributed the decrease in oxygen consumption of the Asian cichlid, *Etroplus suratensis*, to extensive hyperplasia and hypertrophy of gill filaments infested with *Ergasilus ceylonensis*.

Eosinophilic granular cells are common in chronic parasitic infections of the gills (Ferguson 1988). Infiltrations of lymphocytes, eosinophilic granular cells, macrophages and neutrophils accompanied the proliferative response associated with attachment of *E. lizae* Krøyer to the gills of yellowfin bream, *Acanthopagrus australis* (Roubal 1989). Oldewage and van As (1987) described a hollow in the area of proliferated branchial epithelium opposing the mouthparts of *E. mirabilus* attached to the gills of fish, but in Macquarie perch in this study, there was only a mild indentation in the epithelial cushion adjacent to the attached parasite.

Comparison of treatments

During the 1987 breeding trials, while infested fish were held in 2000 L tanks, applications of 10.0 g/L NaCl (60 min.), 5.0 g/L NaCl plus 1.0 mg/L methylene blue (24 hours), and 0.15 mg/L Dipterex® (24 hours), all failed to eradicate *E. intermedius*.

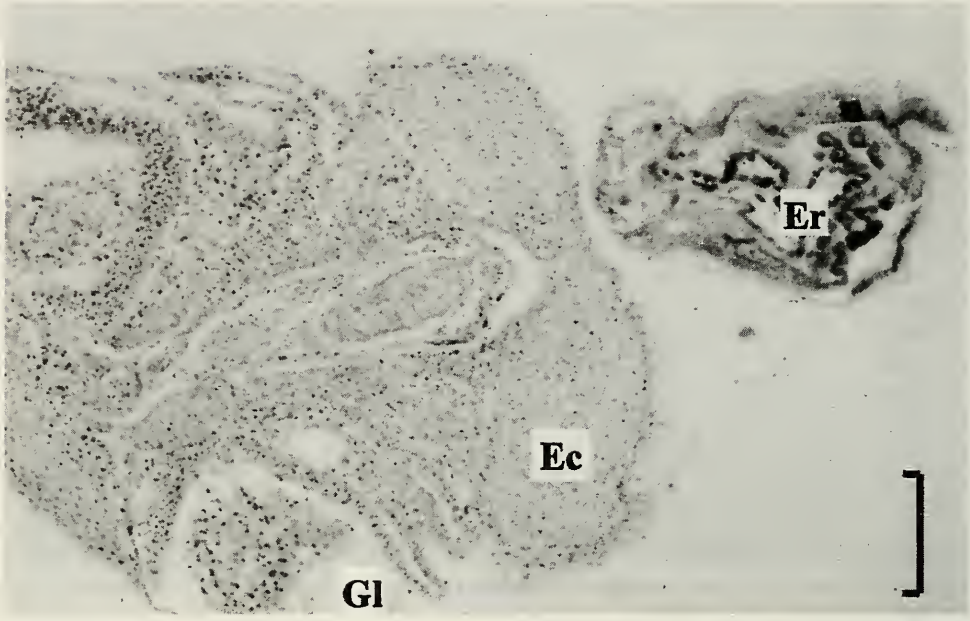

Figure 2. Stained cross section of a gill filament showing cushion of proliferated epithelial cells (Ec) adjacent to the site of attachment by *E. intermedius* (Er), and fused gill lamellae (Gl). (Scale bar length = 125 μm).

However, these fish were subsequently treated with 1.5 mg/L Dipterex® (24 hours) which reduced the infestation by 30% after 24 hours. Three days later all the parasites appeared to be dead.

During the 1988 breeding trials, a single 24 hour treatment of 2.0 mg/L Dipterex® completely eradicated *E. intermedius*, and in 1989, the next time the fish were recovered from the ponds, no fish were parasitised by the ergasilids.

Between 1987 and 1988, ponds containing the Macquarie perch broodfish were treated with malachite green at 0.08 mg/L primarily to control ectoparasitic ciliates, but this treatment was not successful in eliminating *E. intermedius*, as infested fish were recovered from these ponds after treatment.

Previous studies have shown that treatment with Dipterex® was an effective method of controlling ergasilid infestations (Lahav and Sarig 1967; Sarig 1971). Sarig (1971) indicated that the minimum concentration of Dipterex® for killing Ergasilus was 0.15 mg/L. The ineffectiveness of salt treatments suggests that *E. intermedius* is tolerant to the salinity levels and exposure times normally used to treat external parasites on freshwater fishes. Indeed some ergasilids, such as *E. lizae* (Kelly and Allison 1962), are euryhaline.

Records of co-hosts

This is the first time *E. intermedius* has been recorded from Macquarie perch, and represents the most southerly record of this parasite. Kabata (1992) recorded *E. intermedius* from *Maccullochella macquariensis* (Cuvier), the type host, collected from Moreton Bay (Queensland), and *Tandanus tandanus* Mitchell, *Nematalosa erebi* (Günther) and *Macquaria ambigua* (Richardson), all collected from the Macintyre River in Queensland. Kabata (1992) concluded that, having such a wide range of hosts, *E. intermedius* was a successful parasite. The occurrence of *E. intermedius* on Macquarie perch

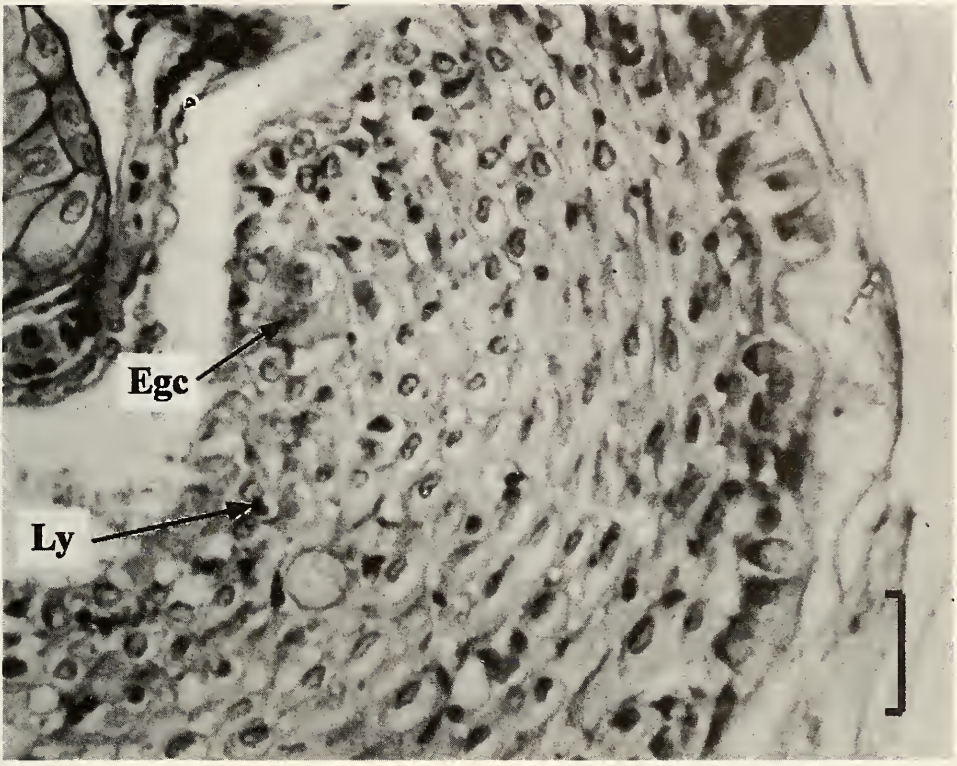

Figure 3. Stained cross section showing a lymphocyte (Ly) and an eosinophilic granular cell (Egc) in the cushion of proliferated epithelial cells. (Scale bar length = 30 μm).

increases the number of known hosts to five, which further demonstrates the success of this species as a parasite. However, we consider that both the identity and the collection site for the type host are incorrect. At the time of collection (1967), *M. macquariensis* was the species name for Murray cod, a relatively common and widespread species endemic to the Murray-Darling River system, including the Macintyre River. In 1972, the species name *M. macquariensis*, was assigned to trout cod, while Murray cod was renamed *Maccullochella peeli* (Berra and Weatherley 1972), then later corrected to *M. peelii peelii* (Rowland 1993). Trout cod is known to have occurred in tributaries of the Macquarie, Murrumbidgee and Murray River systems only (Berra and Weatherley 1972). Therefore the type host is more likely to be Murray cod. Since Moreton Bay is outside the range of Murray cod, the type host may also have been collected from the Macintyre River where the species occurs naturally, and where all other co-hosts of *E. intermedius* were collected.

Because ergasilids have been responsible for massive losses of farmed fish in other parts of the world (Amlacher 1970; Sarig 1971), *E. intermedius* infestation is a potential threat to Macquarie perch held in captivity, as loss of valuable broodfish to outbreaks will hinder conservation efforts. Captive breeding programs have been developed, as a conservation measure, to produce seedstock for release into selected areas to re-establish populations. Loss of broodfish to parasitism reduces the number of seedstock available for release, and can lead to a reduction in genetic variability and a loss of rare alleles. In addition, due to the rarity of this species, replacement of broodfish can be costly and time consuming. Therefore, parasitic outbreaks should be promptly treated to prevent such losses occurring.

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